CLAIMS

1. A method of preparing a proliferation-regulated recombinant adenoviral vector effectively, comprising the steps of:

preparing a proliferation-regulated vector plasmid by preparing a restriction enzyme-recognizing unit in a vector plasmid having a proliferation-regulating unit and having an E1A region, at least one protein-coding region in a E1B region or the entire E1B region, a poly(A) signal sequence, and a recombinase-recognizing sequence in that order from upstream, by deleting both an endogenous promoter in the E1A region and an endogenous promoter regulating expression of the protein-coding gene at least in one protein-coding region of the E1B region and inserting restriction enzyme-recognizing sequences respectively in these deficient sites;

introducing a promoter expressing specifically in a target organ in the restriction enzyme-recognizing unit; and

additionally, integrating the proliferation-regulated vector plasmid into a vector plasmid having an adenoviral genome prepared by deleting the E1 region.

2. The method of preparing a proliferation-regulated recombinant adenoviral vector efficiently according to claim 1, wherein the E1A region lacks a Rb protein-binding sequence.

3. The method of preparing a proliferation-regulated recombinant adenoviral vector efficiently according to claim 1 or 2, wherein the protein-coding region in the E1B region includes a 19KDa protein-coding region and/or a 55KDa protein-coding region.

4. The method of preparing a proliferation-regulated recombinant adenoviral vector efficiently according to any one of claims 1 to 3, wherein each of the restriction enzyme-recognizing sequences inserted to the sites lacking the endogenous promoter in the E1A region and the endogenous promoter regulating expression of the protein-coding gene at least in one protein-coding region of the E1B region has a blunt-end restriction enzyme site

5. The method of preparing a proliferation-regulated recombinant adenoviral vector efficiently according to any one of Claims 1 to 4, wherein the recombinase-recognizing sequence is LoxP, LoxH, or the mutant sequence thereof.

6. A method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently, comprising the steps of: preparing a second therapeutic gene-expressing vector plasmid by allowing a recombinase to react with the proliferation-regulated vector plasmid according to any one of claims 1 to 5 and a first

therapeutic gene-expressing vector plasmid prepared by inserting a constitutive high-expression promoter or a therapeutic gene-expressing promoter and a therapeutic gene in that order from upstream into the restriction enzyme-recognizing sequence of the vector plasmid containing a therapeutic gene-expressing unit, which is prepared by inserting a recombinase-recognizing sequence and a restriction enzyme-recognizing sequence respectively in that order from upstream; and additionally, integrating the second therapeutic gene-expressing vector plasmid into a vector plasmid having an adenoviral genome prepared by deleting the E1 region.

7. A method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently, comprising the steps of: allowing a recombinase to react with the proliferation-regulated adenoviral vector plasmid according to any one of claims 1 to 5 and the first therapeutic gene-expressing vector plasmid prepared by inserting a constitutive high-expression promoter or a therapeutic gene-expressing promoter and a therapeutic gene in that order from upstream to the restriction enzyme-recognizing sequences of the vector plasmid having a therapeutic gene-expressing unit prepared by inserting a recombinase-recognizing sequence and a restriction enzyme-recognizing sequence respectively in that order from upstream.

8. The method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently, according to claim 7, further comprising the steps of: mixing the proliferation-regulated adenoviral vector plasmid and the first proliferation-regulated adenoviral vector plasmid, allowing a recombinase to react with the mixture, and then, transforming the vectors into each other.

9. The method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently, according to claim 7, further comprising the steps of: cotransfecting the proliferation-regulated adenoviral vector plasmid and the first therapeutic gene-expressing vector plasmid to a recombinase-expressing cell.

10. The method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to claim 9, wherein the recombinase-expressing cell is a cell prepared by making an adenoviral E1-region protein-expressing cell additionally express a recombinase.

11. The method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic

gene efficiently according to any one of claims 6 to 10, wherein the recombinase-recognizing sequence in the vector plasmid containing a therapeutic gene-expressing unit is different from the recombinase-recognizing sequence in the vector plasmid having a proliferation-regulating unit.

12. The method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to any one of claims 6 to 11, wherein the drug tolerance gene in the vector plasmid having a proliferation-regulating unit and the drug tolerance gene of the vector plasmid having a therapeutic gene-expressing unit are different from each other, and Ori in the vector plasmid containing a therapeutic gene-expressing unit can duplicate pir genes such as R6Kγ only in competent cell.

13. A vector plasmid having a proliferation-regulating unit, for use in the method of preparing a proliferation-regulated recombinant adenoviral vector efficiently according to any one of claims 1 to 5.

14. A preparative kit, including a vector plasmid having a proliferation-regulating unit and a vector plasmid having an E1 region-deficient adenoviral genome, for use in the method of preparing a proliferation-regulated recombinant adenoviral vector efficiently according to any one of claims 1 to 5.

15. A vector plasmid having a therapeutic gene-expressing unit, for use in the method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to any one of claims 6 to 12.

16. A preparative kit, comprising a vector plasmid having a proliferation-regulating unit, a vector plasmid having a therapeutic gene-expressing unit, and a vector plasmid having an adenoviral genome at least lacking the E1A region, for use in the method of preparing a proliferation-regulated recombinant adenoviral vector having an integrated therapeutic gene efficiently according to any one of claims 6 to 12.

17. A treatment method for various diseases including malignant tumors, wherein the proliferation-regulated recombinant adenoviral vector prepared by the method according to any one of claims 1 to 12 is utilized.